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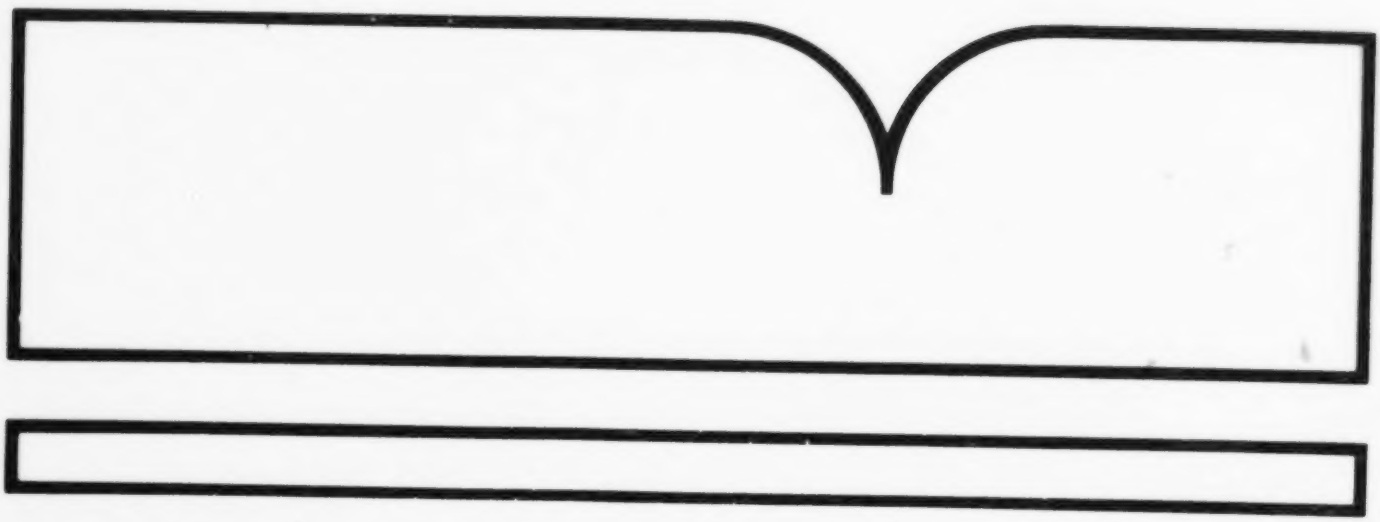

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APPLICATION OF ADENINE NUCLEOTIDE MEASUREMENTS FOR THE EVALUATION OF STRESS IN *MYTILUS EDULIS* AND *CRASSOSTREA VIRGINICA*

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Abstract—1. After 10 weeks treatment with 10 μg Ni/kg seawater, the concentration of ATP in *Mytilus edulis* adductor muscles was significantly less than that in muscles from control and 5 μg Ni/kg treated mussels.

2. Mussels sampled in August after exposure for 12 weeks to polluted and unpolluted waters had significantly lower AEC, ATP/ADP and ATP/AMP ratios than those sampled in May from the same locations.

3. The percentage composition of the individual adenine nucleotides in the adenylate pool appears to be directly related to the amount of stress in *Mytilus edulis* and *Crassostrea virginica*.

4. The degree of change in AEC which constitutes a significant biological effect remains to be established.

INTRODUCTION

All too often, the lack of an alternative dictates the use of marine organisms assumed to be "healthy" in studies which measure responses of these organisms to environmental perturbations. These assumptions of "well being" are usually based on available knowledge of the environmental conditions at the collection sites. The need for a biochemical or physiological indicator of "well being" is pressing since knowledge of the physiological condition prior to testing would lend credibility to comparison of results of testing performed during different seasons. In addition, a physiological or biochemical indicator of stress would be valuable in determining sublethal effects of toxicants.

Two of the more promising indicators of physiological condition are "Scope for Growth" (Bayne *et al.*, 1976) and "Adenylate Energy Charge" (Ivanovici, 1974; Wiebe & Bancroft, 1975). Consideration of each method suggested that the adenylate energy charge (AEC) offers several advantages over "Scope for Growth". These included smaller sample size, independence of feeding behavior during the test and greater precision, i.e. lower variability between each test organism.

Atkinson & Walton (1967) proposed AEC as a measure of energy potentially available from the adenylate system for cell metabolism. The AEC defined as (ATP + 1/2 ADP)/(ATP + ADP + AMP), has a maximum value of 1.0, when all adenylate is in the form of ATP, and a minimum value of 0 when all adenylate is in the form of AMP (Atkinson & Walton, 1967). The energy charge is considered important in the control of key catabolic and anabolic pathways (Atkinson, 1971). Values of energy charge correlate with physiological condition: energy charges between 0.8 and 0.9 are typical of organisms which are actively growing and reproducing usually under optimal environmental conditions (Chapman *et al.*, 1971; Atkinson, 1971; Rainer *et al.*, 1979; Ivanovici, 1980a). Values in the range of 0.5 to 0.7 have been observed in organisms which are stressed (Ball & Atkinson, 1975; Behm & Bryant, 1975; Wijsman, 1976; Rainer *et al.*, 1979; Ivanovici, 1980a) and whose growth and reproductive rates are reduced (Chapman *et al.*, 1971). Values below 0.5 have been associated with irreversible loss of viability under detrimental conditions (Ridge, 1972; Montague & Dawes, 1974; Skjoldal & Bakke, 1978).

In this study we evaluated changes in nucleotide metabolism as indices of stress in the mussel (*Mytilus edulis*) and the American oyster (*Crassostrea virginica*).

MATERIALS AND METHODS

Laboratory Study: Oysters (*Crassostrea virginica*) were obtained from Cotuit, MA, U.S.A., in April 1979 and mussels (*Mytilus edulis*) were collected from an unpolluted area in Narragansett Bay, RI, U.S.A., in April 1979. The oysters and mussels were acclimatized for about 4 weeks in fiberglass troughs (3.75 m long × 30 cm wide × 25 cm deep), supplied with unfiltered Narragansett Bay seawater (29–32‰ salinity) at a rate of 20 l/min. The oysters and mussels were placed on polyethylene grids raised 2.5 cm above the bottom of the troughs. Since the incoming seawater contained ample food organisms, the oysters and

Table 1. Sample size determination for the detection of fixed magnitudes of differences in AEC, ATP/ADP and ATP/AMP for *Mytilus edulis* after being held in Narragansett Bay for 4 weeks (May, 1979)

Variable	Magnitude of difference	$x^* \pm 50\%$	Variance	N†
AEC	0.1		0.00068	1
ATP/ADP	$x \pm 50\%$	3.48 ± 1.74	0.73	3
ATP/AMP	$x \pm 50\%$	7.58 ± 3.79	3.03	3

* x = The overall mean; the power of the test = 0.80; $P = 0.10$.
† N = The calculated sample size needed to detect a 50% change in the mean value of 0.1 unit change in AEC.

mussels were not fed during the acclimatization or treatment periods. A deionized water solution of nickel chloride was metered into the troughs (3 for each concentration) to produce concentrations of 5 and 10 μg Ni/kg when mixed with incoming seawater which contained 1–2 μg Ni/kg. No mortalities were observed in the control or experimental troughs throughout the study.

At the end of 10 weeks (June 1979 to August 1979) six oysters and six mussels were removed from each treatment and the adductor muscles from each were dissected and prepared individually for adenylate analyses according to the procedures of Ivanovici (1980a,b).

Field study

In April 1979, *Mytilus edulis* were collected from the same site as that used for the laboratory study. Twenty mussels were placed in each of several plastic baskets (15 cm^3) and suspended in the water column at two stations in Narragansett Bay (station numbers 2 and 4 of the Coastal Environmental Assessment Station (CEAS) program, Phelps & Galloway, 1979) at which earlier physiological studies had been performed (Widdows *et al.*, 1980). Station 2 is closer to industrial and urban areas than station 4, and the water is impacted primarily by sewage treatment effluent. Station 4 was chosen as the control site. In May and August, 1979, (representing 4 and 12 weeks holding) samples of mussels were removed from each station and the adductor muscle tissue from each mussel was analyzed for adenylates according to the procedures of Ivanovici (1980a,b). In addition, the size, reproductive condition (unspawned, partially or fully spawned) and handling time (time from opening to freeze clamping) were recorded when animals were sampled.

Differences in station and laboratory treatment means were analyzed by analyses of variance and covariance with square root and log transformations applied where necessary to reduce the variance. Scheffe's multiple comparison procedure was used to group population means when the analyses of variance indicated a significant difference among population means (Scheffe, 1953). Sample size determinations were based upon a predetermined type I error of 0.10 and a type II error of 0.20 with a fixed difference of 0.1 unit for AEC and a 50% change in the mean value for other measured variables such as ATP/ADP and ATP/AMP. Whether a 50% change in the mean is biologically significant is uncertain.

RESULTS

Mussel study

Since optimal sample sizes for the determination of adenine nucleotides in several molluscan species (including *M. edulis*) vary from three to eight in the literature (Wijsman, 1976; Rainer *et al.*, 1979; Ivanovici, 1980a), determinations of optimal sample size were performed on the combined data from stations 2 and 4 in May. Twelve mussels were processed from each station (one mussel lost from station 4). These determinations indicated that a sample size of three was adequate and that of eight was more than adequate to detect significant differences for the variables AEC, ATP/ADP and ATP/AMP (Table 1).

Linear regression analyses which were performed on the combined field and laboratory data to check for effects of handling time, size or reproductive condition on the adenine nucleotides showed only a significant effect of handling on ADP concentrations and a significant size effect on ATP. Reproductive condition had no significant effect on any of the nucleotides. Analysis of covariance was used if handling time, size or reproductive condition was a significant factor.

Table 2. The response of adenine nucleotides in the muscle tissue of *Mytilus edulis* after treatment with ambient (control), 5 and 10 μg Ni/kg seawater at ambient temperature and salinity for 10 weeks

Treatment	Sample size	AEC	μmol/g wet weight ATP	ADP	AMP	ATP + ADP + AMP	ATP/ADP	ATP/AMP
Control	6	0.85*	2.82†	0.97	0.09	3.89	3.00	38.94
		0.01	0.12	0.10	0.03	0.20	0.23	7.51
5 μg Ni/kg	6	0.80	2.72†	1.10	0.21	4.03	2.62	30.54
		0.05	0.34	0.08	0.10	0.20	0.42	9.55
10 μg Ni/kg	6	0.75	1.84†	1.02	0.23	3.09	1.83	12.80
		0.04	0.26	0.07	0.07	0.25	0.26	3.31

* Mean value of each sample with standard error below.
† Mean adjusted for covariate size.

Table 3. The response of adenine nucleotides in the muscle tissue of *Mytilus edulis* after being held at the respective stations in Narragansett Bay for 4 (May) and 12 weeks (August)

Time and treatment	Sample size	AEC	ATP	ADP	AMP	ATP + ADP + AMP	ATP/ADP	ATP/AMP
				μmol g wet wt				
May								
Station 4 (control)	11	0.88	3.50*	0.96	0.06	4.51	1.93	8.31
		2×10^{-4}	0.12	0.06	0.01	0.15	0.06	0.47
Station 2	12	0.86	3.66	1.22	0.10	4.98	1.77	6.86
		1×10^{-3}	0.21	0.12	0.02	0.25	0.08	0.54
August								
Station 4 (control)	6	0.70	2.69	1.30	0.60	4.60	1.36	3.08
		0.09	0.65	0.18	0.33	0.66	0.14	0.63
Station 2	4	0.65	1.45	1.37	0.52	3.35	1.03	1.82
		0.03	0.14	0.14	0.14	0.36	0.05	0.26

* Mean value of each sample with standard error below.

Laboratory

The concentration of ATP in mussels treated with 10 μg Ni/kg was significantly less ($P < 0.05$) than control and 5 μg Ni/kg treated mussels (Table 2). The adenylate pool was significantly reduced ($P < 0.05$) in the 10 μg Ni/kg treatment. The differences between the adenylate pools in control mussels and those in the 5 or 10 μg/kg treatment were not significant ($P > 0.05$), although the difference between the 5 and 10 μg Ni/kg treated mussels was. Analyses of covariance with size and reproductive condition as covariates detected no significant effects on concentrations of ADP and AMP.

Analysis of variance indicated significant treatment effects on the ATP/ADP and ATP/AMP ratios ($P < 0.05$). The differences between the controls and 10 μg Ni/kg treatments for ATP/ADP and ATP/AMP were significant ($P < 0.05$). Differences between AEC's of control and treated mussels were not significant, although a decreasing trend is apparent.

Since the AEC showed non-significant treatment differences, apparently due to higher variability than expected, a determination of the sample size necessary to detect fixed differences between the means for ATP/ADP and ATP/AMP indicated that sample sizes of 5 and 8 respectively are needed to detect a 50% change per treatment. Eleven mussels are needed to detect 0.1 unit change in AEC.

Field

Since handling time and size did not contribute significantly to the total variance ($P > 0.05$; analysis of covariance), the data were not adjusted for these factors. Significant effects were found for the individual nucleotides and the adenylate pool (Table 3).

Differences between means were not significant for ATP, ADP, AMP and the adenylate pool in mussels sampled from stations 4 and 2 either after 4 weeks (May) or after 12 weeks (August) (Scheffé's Test, $P > 0.05$). The concentration of AMP was significantly higher in mussels sampled at station 4 in August than in May. At station 2, the concentration of ATP and the adenylate pool was less in August than in May, while that of AMP was significantly higher.

The interaction between station and date (seasonal effect) was not significant ($P > 0.05$). This allowed pooling of the data from stations 2 and 4 for May and August respectively to establish an effect of time on the concentration of the adenine nucleotides. The subsequent analysis of the May and August data indicated that time has a significant ($P < 0.05$) effect on the concentration of ATP and AMP. In addition, differences between mean AEC, ATP/ADP and ATP/AMP were significant ($P < 0.05$) between May and August samples for stations 4 and 2 examined individually and pooled (Table 3). No significant differences in AEC, ATP/ADP and ATP/AMP were obtained in mussels between stations in May or August.

A determination of the sample size necessary to detect fixed differences between the means indicated that the sample size used in the field study was not large enough. In order to detect a 50% change in the mean per treatment for ATP/AMP, sample sizes of 8 and 11 respectively are required. Thirty-two mussels are needed to detect a 0.1 unit change in AEC.

To further evaluate the use of adenine nucleotides as indicators of stress in *M. edulis*, changes in the concentrations of the individual nucleotides within the pool expressed as per cent total were determined for both the laboratory and field studies. The magnitude of change in concentration of the individual adenine nucleotides within the pool was directly related to the amount of stress (Fig. 1). As the amount of stress increased, the concentration of ADP and AMP as per cent of total adenylate increased while that of ATP decreased in all concentrations.

Oyster study

The responses of the concentrations of ATP, ADP, AMP and the adenylate pool of the adductor muscles of *Crassostrea virginica* to the effect of ambient (control), 5 and 10 μg Ni/kg seawater are presented in Table 4.

Linear regression analyses which were performed to determine the effects of handling time, size and reproductive condition on the nucleotide variables, indicated a significant ($P < 0.05$) linear relationship between size and ATP, ADP and adenylate pool. A significant ($P < 0.05$) relationship also existed

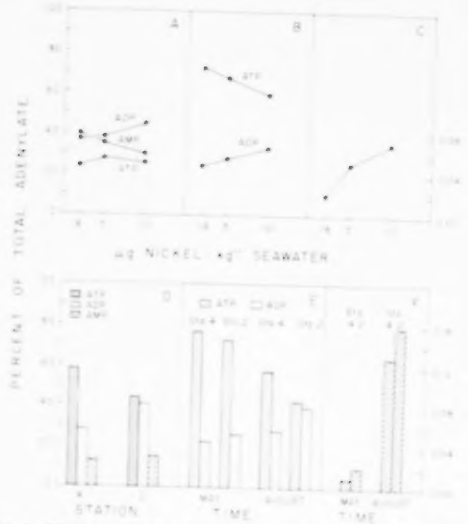

Fig. 1. Changes in adenylate concentrations in response to environmental perturbations. A. *Crassostrea virginica*. B,C. *Mytilus edulis*. After holding in flowing ambient seawater (29–32‰ salinity) containing 1.8 (ambient), 5 or 10 μg Ni/kg for 10 weeks. D. The effect of environmental conditions at station 2 (polluted) and station 4 (unpolluted) on the concentration of ATP, ADP and AMP in *M. edulis* in August after 12 weeks. E,F. The effect of time (12 weeks) on the concentration of ATP, ADP and AMP in *M. edulis* held at stations 2 and 4.

between reproductive condition and AMP. However, no significant relationships existed among the other variables.

Analyses of covariance with size as a covariate were performed on the transformed values of Table 4 for ATP, ADP and the adenylate pool to adjust these values for the effect of size. Analysis of variance was performed on the transformed AMP values since size had no significant relationship with AMP. The apparent difference in AMP between the control and the 10 μg Ni/kg treatment (Table 4) is not significant due to high variance and small sample size. Results are similar for the analysis of covariance on the adenylate pool.

Although correction for size increased the precision of the data, the small sample size and high variability prevented detection of significant treatment effects of nickel on the oyster ATP or ADP.

Inclusion of the covariates was responsible for a significant gain in precision of the error variance and indicated that treatment effects were not explained by differences in size, handling time or reproductive condition. Thus, treatment differences (or lack of difference) cannot be attributed to any difference that might exist between the groups for the variables, size, reproductive condition and handling time.

Despite the lack of significant treatment effects on the AEC and the adenine nucleotides, a relationship was apparent when the concentration of the individual adenine nucleotides was expressed as a percentage of the adenylate pool (Fig. 1). In all treatments, the concentration of ADP was always greater than ATP in the oyster muscle tissue. The highest concentration of ADP and lowest concentration of ATP were found in the 10 μg Ni/kg treatment. In addition, the AMP concentration decreased with increased nickel concentration.

DISCUSSION

Although small sample sizes (three to eight individuals) have been adequate to detect statistically significant differences in earlier laboratory and field studies (Giesy *et al.*, 1978; Rainer *et al.*, 1979; Ivanovici, 1980a), the present field study showed that six mussels per sample were inadequate. It is not uncommon to experience greater variability under field conditions than under laboratory conditions (Lynch, 1974). These data contrast with findings of AEC for gastropod and bivalve species sampled from field conditions (Ivanovici, in prep.; Rainer *et al.*, 1979).

Ivanovici (1980a) showed that transferring *Pyrazus ebininus* from their natural environment to laboratory conditions affected neither the AEC nor any other nucleotide variable significantly. Rainer *et al.* (1979) reported no laboratory-induced effects on the clam, *Anadara trapezia* and the oyster *Saccostrea commercialis*. Studies by Simpson (1979) indicate that *M. edulis* may be physiologically affected by laboratory conditions. Our data support that of Ivanovici (1980a) in that after 10 weeks holding in the laboratory, no significant changes in AEC or any variable were observed in *M. edulis*. This indicates that *M. edulis* could be used successfully for experiments over long periods under laboratory conditions reported in this study.

In many environmental studies, control of all the factors that influence a biological response and contribute to its variability is difficult. Therefore, CEAS stations (Phelps & Galloway, 1979) where environ-

Table 4. The response of adenine nucleotides in muscle tissues of *Crassostrea virginica* after treatment with ambient (control), 5 and 10 μg Ni/kg seawater at ambient temperature and salinity for 10 weeks

Treatment	Sample size	AEC	μmol/g wet weight				ATP/ADP	ATP/AMP
			ATP	ADP	AMP	ATP + ADP + AMP		
Control	6	0.42*	0.94	1.49	1.59	4.01	0.51	1.04
		0.10	0.37	0.45	0.36	0.65	0.15	0.38
5 μg Ni/kg	6	0.47	0.86	1.19	1.08	3.13	0.68	1.40
		0.09	0.21	0.20	0.37	0.20	0.18	0.44
10 μg Ni/kg	6	0.46	0.75	1.33	0.87	2.96	0.54	1.03
		0.06	0.21	0.18	0.10	0.32	0.14	0.38

* Mean value of each sample with standard error below.

mental conditions have been monitored for a considerable period were selected. In addition, physiological studies on *M. edulis* transferred to these stations had been done a year earlier (Widdows *et al.*, 1981).

Linear regression analyses performed to assess possible effects of handling time, size and reproductive condition on the nucleotide variables, indicated no effect on samples collected in May. In the August samples, however, concentrations of ADP in the mussels were significantly ($P < 0.05$) affected by handling time. The regression analyses indicated that large mussels have significantly ($P < 0.02$) higher concentrations of ATP than do smaller mussels. Since small *M. edulis* respire at higher rates than larger ones (Read, 1962), higher concentrations of ATP were expected in small mussels to support the higher metabolic activity. The low ATP concentration in the small mussels suggests that either ATP was not regenerated at a sufficiently fast rate to meet the metabolic demands of the small mussels or that it was consumed at such a fast rate as to prevent accumulation, particularly in August when seawater temperatures are at their highest (19–20 C). Lower mean seawater temperatures (10–12 C) and lower metabolic rates in mussels during May than in August could have contributed to the lower individual variability and reduced sample size estimates obtained with the May sample.

Significant treatment effects were not found for concentrations of AMP. This may have been due to the large variability associated with this nucleotide. The low variability of AEC values and the high variability of the nucleotide variables obtained in this study are consistent with that of previous studies (Ballard, 1971; Ching *et al.*, 1975; Ivanovici, 1980a).

Although a 0.1 change in AEC was not significant in this study, there were indications that the mussels were physiologically stressed. The ATP/ADP ratio, which is considered to reflect the metabolic status of the cell (Beis & Newsholme, 1975; Harpold & Craig, 1975), was significantly less in mussels from the 10 μg Ni/kg treatment than the controls. In addition, the ATP/ADP ratio was significantly less in mussels at station 2 than that in mussels from station 4 in August. This indicated that reduced metabolism was concurrent with a stress condition. Physiological stress has been demonstrated in *M. edulis* during and after spawning (Widdows, 1978). Thus from Widdows' (1978) data, the mussels used in the August sample, which were either partially or completely spawned, were probably undergoing stress associated with reproductive condition. Regression analyses, however, indicated that reproductive condition did not contribute to the overall variability of the adenine nucleotide variables. Thus, the possible stress effect on nucleotides due to spawning can be discounted here, but experiments specifically aimed at examining changes in adenine nucleotides at various and specified times during the reproductive cycle are in order.

Further indications of a stress condition were provided by histopathological and mortality data. Tissue from mussels sampled from station 2 had extensive amoebocytic infiltration and necrosis, both indicative of poor condition, while mussels from the control site (station 4) showed no pathology (P. Yevich, personal communication). Few mortalities occurred at station 4, in marked contrast with station 2, where less than 20% of the mussels survived.

Reproductive condition did not appear to significantly alter the response to stress by the AEC, since a difference in AEC for nickel treatments (significant between the control and the 10 μg/kg treatments) was obtained in mussels during the spawning period. The majority of the mussels in the laboratory study were partially spawned with the remainder completely spawned (in all three treatments) at the time of the August sample. This strengthens the assumption that stresses can be detected in mussels with use of AEC, independent of reproductive condition.

In the field, factors such as water quality and crowding in the baskets may have affected the condition of the mussels. The lower AEC value (0.70) obtained with mussels from station 4 in August compared to 0.85 measured in laboratory controls, suggests that laboratory conditions may have been better for the mussels than the ambient condition in the experimental containers. This has been found in oysters (Rainer *et al.*, 1979). It was not clear how water quality contributed to the apparent stress of the mussels at station 4, since the seawater used for the laboratory studies was taken from station 4. If the biomass per basket were too high, non-optimal conditions may have been present for the mussels. The effect of different population densities on nucleotides remains to be checked. *Mytilus edulis* close their valves when environmental conditions deteriorate and the anaerobic condition created by shell closure causes a reduction in metabolic demand (de Zwaan & Wijsman, 1976; Wijsman, 1976). Thus, shell closure in *M. edulis* is reflected by a reduction in AEC (Wijsman, 1976). Shell closure may explain the low AEC values obtained with mussels from station 4 in August, but this was not verified by observation.

Changes in adenylate pool were not consistent with lower AEC values. For example, at station 4 in August, the lower AEC was not accompanied by a reduced adenylate pool. The per cent composition of the adenylate pools which was determined for mussels from the laboratory and field studies indicated a clearer relationship between treatments and adenine nucleotides than did the absolute concentrations and the AEC. We feel that the AEC did not accurately reflect the apparent severe metabolic stress in the mussels at station 2 in August, since a significant decrease in the adenylate pool of these mussels prevented a greater decrease in the AEC value than that obtained. In order that changes in AEC be related to physiological condition, the adenylate pool must remain constant. If the pool decreases consistently with stress in the species studied, then that species may be unsuitable for AEC measurements (Atkinson, 1968). After treating the isopod, *Cirolana borealis*, with toluene, Bakke & Skjoldal (1979) reported that ATP is a more sensitive indicator of stress than AEC and concluded that concentrations of adenine nucleotides and AEC are not suitable as indicators of stress in environmental pollution studies.

Oyster study

The low AEC values measured in oysters indicated that they were severely stressed to the point of losing

viability, even after transfer to non-stressful conditions. Adenylate energy decreases to 0.4 in the marine isopod *C. borealis* after being killed with toxic concentrations of toluene (Bakke & Skjoldal, 1979). In contrast, an AEC value of 0.72 was reported for dead *M. edulis* (Wijsman, 1976); however, bacterial growth occurring in dead mussel tissue could contribute to this high value.

Values of AEC less than 0.8 have been measured in actively growing organisms (Zs-Nagy & Ermini, 1972; Eigener, 1975). High AEC values have been measured in organisms which were known to be under lethal conditions (Chapman & Atkinson, 1973; Ivanovici, unpublished data), suggesting that the method may not be suitable for some species. The low AEC coupled with the decrease in adenylate pool indicate that AEC is probably unsuitable as a measure of stress in *C. virginica*. Low AEC values and changes in total adenylates also occur in the oyster, *S. commercialis* (Rainer *et al.*, 1979), due possibly to sampling and handling effects. It is possible that the oysters in this study were severely stressed from conditions other than the nickel treatment, since significant treatment affects were not observed ($P > 0.05$). For example, laboratory conditions may have affected the nucleotides of *C. virginica*.

In addition, since the oysters were either partially or completely spawned, the stress imparted by the spawning process may have contributed to the low AEC values. That all the oysters were either partially or completely spawned might explain why the analyses of covariance did not indicate a significant effect of reproductive condition on AEC. Oysters in all treatments appeared to be equally stressed (as indicated by the low AEC). However, changes in the ATP and ADP concentrations among treatments were consistent with stress conditions (ADP concentrations were consistently higher than ATP concentrations). Inconsistent with stress conditions was the change in AMP concentrations with respect to ATP among treatments. Contrary to what we had expected, decreases in ATP concentrations were accompanied by decreases in AMP concentrations. Ivanovici (1980a) reported high AMP concentrations concidental with low ATP concentrations in the estuarine mollusc *P. ebeninus* when stressed. Wijsman (1976) reported similar findings when *M. edulis* was stressed.

Despite the implications of the low AEC value, the oysters remained alive and viable with no indications otherwise (these oysters were held for 40 weeks in flowing ambient seawater in our system). The well being of these oysters was confirmed by histopathological examination (P. Yevich, personal communication). The key may lie in the role of AMP, since AMP concentrations did not follow the pattern reported for other stressed invertebrates (Hiltz & Dyer, 1970; Zs-Nagy & Ermini, 1972; Wijsman, 1976; Ivanovici, 1980a).

CONCLUSION

Several studies have examined AEC values in several marine molluscs under a variety of environmental conditions (Ivanovici, 1974, 1980a; Wijsman, 1976; Rainer *et al.*, 1979) and it has been demonstrated that the AEC value varied consistently with perturbations in environmental conditions. We have clearly shown this to exist in the laboratory study with mussels but less clearly in the field study. However, what degree of change in AEC constitutes a significant biological effect remains to be established. Values of AEC obtained with *C. virginica* are indicative of imminent death (Skjodal & Bakke, 1978). However, the fact that oysters in this study were successful suggests that the implications of a lowered AEC need to be studied more closely for individual species in order to assess the physiological significance of lowered AEC values.

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